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CASE DB13NP

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Keith R. Lange
Type or print name

Signature

December 4, 2002
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
STARLING ET AL.
APPLICATION NO: 09/745,605
FILED: DECEMBER 22, 2000
FOR: NOVEL IMMUNOGLOBULIN SUPERFAMILY MEMBERS OF APEX-1, APEX-2 AND APEX-3 AND USES THEREOF

Art Unit: 1644
Examiner: Haddad, Maher M.

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF PRIOR INVENTION IN THE UNITED STATES TO OVERCOME A REFERENCE UNDER 37 C.F.R. § 1.131

Sir:

1. We, Gary C. Starling and Joshua N. Finger, both citizens of the United States, residing respectively at 11 Gallo Court, Lawrenceville, NJ 08648 and 197 North Union Street, Apt. B, Lambertville, NJ 08530 are joint inventors of the above-identified application.

2. At the time of the invention thereof we were working for Bristol-Myers Squibb Company, assignee of the present application. We submit this declaration to establish completion of the invention set forth in this application in the United States at a date prior to December 9, 1999, i.e. the publication date of WO 99/63088 to Baker et al. (hereinafter the '088 publication), which was cited by the Examiner in an Office Action mailed June 5, 2002.

3. From the documents submitted herewith and as set forth hereinbelow, it can be seen that the invention was completed in the United States before December 9, 1999, the publication date of the '088 publication. Completion of the invention prior to December 9, 1999 is shown by conception and actual reduction to practice of the invention as evidenced by the cloning and sequencing of the APEX-1 gene (hereinafter "APEX-1"), which is also referred to in Exhibit A as DCS4.

4. To establish conception and reduction to practice, i.e. completion of the invention at a date prior to December 9, 1999, the following documents are submitted as evidence:

a. Bristol-Myers Squibb Notebook No. 42973 assigned and completed prior to December 9, 1999 (Exhibit A), pages 42973-103 through 42973-106, 42973-112 through 42973-114, 42973-127 through 42973-129 and page 42973-158. These pages show the full-length cloning of APEX-1 and set forth the nucleotide and amino acid sequences of APEX-1, which correspond to SEQ ID NOS. 1 and 4, respectively, in the present application. The full length cDNA sequence and amino acid translation of APEX-1 is shown on page 42973-158. These notebook records evidence conception and actual reduction to practice of the complete invention prior to December 9, 1999.

5. The materials submitted herewith establish that the invention as claimed was completed, i.e. conceived and reduced to practice, at a date prior to December 9, 1999, the publication date of the '088 publication.

6. This declaration is submitted in a response to a non-Final Office Action dated June 5, 2002 and is therefore believed to be timely filed.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED:_____ _____
Gary C. Starling

DATED:_____ _____
Joshua N. Finger

EXHIBIT A

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

BRISTOL-MYERS SQUIBB

NOTEBOOK Nº 42973

Assigned to Joshua N. Jaeger

Department Name _____

Department Number _____

Date Assigned _____

Date Completed _____

Pages Completed from 001 to 200

Continued from Notebook Number _____

Continued in Notebook Number _____

This notebook cannot be transferred to another person

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* USE THE MOST APPROPRIATE DESCRIPTION, E.G.: COMPOUND NUMBER, PRODUCT NAME AND LOT NUMBER, GENERIC NAME, CHEMICAL NAME, CULTURE NUMBER.

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* USE THE MOST APPROPRIATE DESCRIPTION, E.G.: COMPOUND NUMBER, PRODUCT NAME AND LOT NUMBER, GENERIC NAME, CHEMICAL NAME, CULTURE NUMBER.

DATE PROJ. NO. EXPT. NO.

SUBJECT Isolation of Clone #4 and Clone #82

To isolate the cDNA inserts from clone #4 and clone #82 from plasmid DNA for use as a probe.

Materials and Methods

a) Clone #4

	0.5ml
React III	0.1ml
EcoR1	1ml
dH_2O	3ml
	10ml

b) Clone #82

	19ml
React III	3ml
PstI	1.5ml
HindIII	1.5ml
dH_2O	5.0ml
	30.0ml

Results:

[redacted]

Lane 1: λ/Ø Marker
Lane 2: Clone #4
Lane 3: ~~Clone~~ BLANK LANE
Lane 4 BLANK LANE
Lane 5 Clone #82
Lane 6 Clone #82

Discussion Clone #4 must have a mutated EcoR1 site, cut again with HindIII and PstI. Go ahead and isolate clone #82 insert and analyze.

END

SIGNED DATE WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DO NOT WRITE IN THIS MARGIN

NO. 42973 104

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT Redigest of Clone #4 using PstI or HindIII

PURPOSE To drop out insert of clone #4 using enzymes other than EcoRI

Materials and Methods

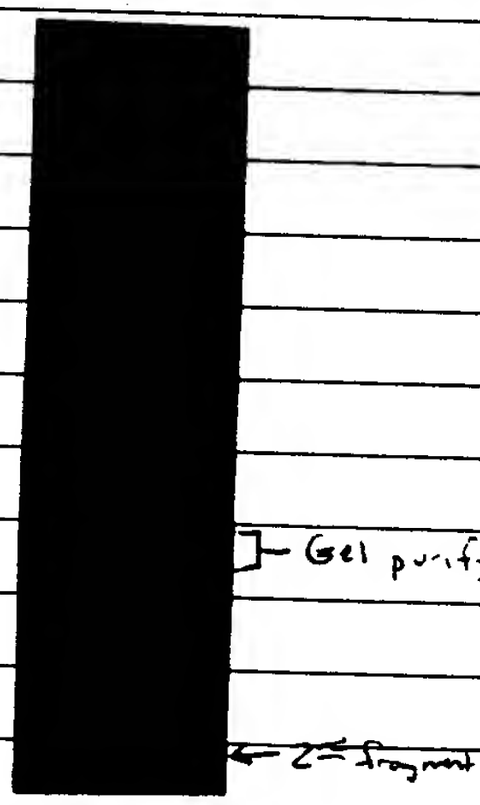
Clone #4	1.5 ul
Buf II	3 ul
PstI	1.5 ul
HindIII	1.5 ul
dH_2O	9.0 ul
	30.0 ul

Results.

Lane 1 Φ marker

Lane 2 H/P digest of DSS4

Lane 3 H/P digest of DSS4



Discussion

Just as I suspected DSS4 clone has a mutated EcoRI site as shown by inability of EcoRI to cut out insert (42973-103, Lane 2). However, HindIII & PstI dropped out two fragments. One fragment approximately 400 bp in size will be gel purified and used as a probe (HP400). The 2nd fragment is approximately 80-90 bp and will go into the trash.

END

SIGNED John M. Jay DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: Hagbe DATE

DATE PROJ. NO. EXPT. NO.

SUBJECT Gel purification of clones #5 and clone #4

PURPOSE To get purity both clones isolated from Dendritic cell subtraction library for use as probes in further expt.

5

Materials & Methods

See protocol from QIAquick Spin Handbook "QIAquick gel extraction kit" (QIAGEN Cat # 28704)

10

RESULTS

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1)	0.0048	0.0027	0.0002	1.8457	0.5418	0.0025	23.1278
2)	0.0109	0.0045	-0.0006	2.2434	0.4457	0.0051	57.5225
3)	0.3912	0.2485	0.0023	1.5793	0.6332	0.2463	1944.6359
4)	0.4535	0.2929	0.0039	1.5558	0.6428	0.2890	2248.1201

$[\,] = (A_{260} \times (50^{ug}/_{ml}) \times D) / 1000 = \frac{ug}{ul}$

1) DSS4

25

$(0.0048 \times 50^{ug}/_{ml} \times 25) / 1000 = \boxed{6^{ng}/_{ml}}$

2) DSS82

$(0.0109 \times 50^{ug}/_{ml} \times 25) / 1000 = \boxed{13^{ng}/_{ml}}$

3) MAXIPREP DSS4 3) DSS4.1 $(0.3912 \times 50^{ug}/_{ml} \times 200) / 1000 = \boxed{3.9^{ug}/_{ml}}$

4) DSS4.2 $(0.4535 \times 50^{ug}/_{ml} \times 200) / 1000 = \boxed{4.5^{ug}/_{ml}}$

DISCUSSION:

35 NONE

END

SIGNED Jan M. Jin DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY Hasse DATE

NO. 42915-100

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE: 01, _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT Southern Hybridization to confirm expression of DSS4 in subtracted library

Purpose: To analyze the presence of DSS4 in DI2 cDNA, THP1 cDNA and DI2-THP1 subtracted cDNA. To also see if other housekeeping genes such as G3PDH were subtracted out of the subtracted cDNA library.

Materials and Methods:

10ug of each cDNA was loaded into each well and ran @ 70Volts for 3 hours. DNA was stained (like gel photo)

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1)	0.6093	0.4039	0.0198	1.5350	0.6515	0.3840	2947.4448
2)	0.9419	0.5982	0.0306	1.6055	0.6229	0.5676	4556.4517
3)	0.2023	0.1188	0.0005	1.7056	0.5863	0.1183	1008.9186

[ ] = Abs x 50 ug/ml x D = ug/ml

1) DI2 cDNA:

$0.4039 \times 50^{ug}/_{ml} \times 50 = 1.5^{mg}/_{ml}$

2) THP1 cDNA: $0.9419 \times 50^{ug}/_{ml} \times 50 = 2.4^{mg}/_{ml}$

3) DI2-THP1 subtracted cDNA: $0.2023 \times 50^{ug}/_{ml} \times 50 =$ c.5 ug/l

[redacted]

~~RT~~ Lane 1 : DI2 cDNA

Lane 2 : THP1 cDNA

Lane 3 : DI2-THP1 subtracted cDNA

SIGNED _____ DATE _____

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY _____ DATE _____

DATE: _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT: 3' RACE using DSS4 gene specific primer and QT, QI, QO

Purpose: To amplify the remaining 3' end of the unknown DSS4 gene which shows homology to CD84

Materials and Methods

1) cDNA synthesis on Dendritic, THP1, and Jurkat cells

poly A+ RNA ~~DNA~~ 2ul
Primer QT(JNF3) 1ul
dH2O (DEPC) 9ul
12ul

*Incubate @ 70°C for 10min. Ice 1min.

Add to RNA/primer mix:

10x PCR Buffer 2ul
25mM MgCl2 1ul
10mM dNTP's 2ul
0.1M DTT 2ul
7ul

Incubate @ 42°C 5min
Add 1ul Superscript II RT
Incubate @ 42°C 1 hour
Stop Rxn by 70°C incubation
Add 1ul RNase H
Store @ -20°C

continued 113

SIGNED: [signature] DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: [signature] DATE

DATE PROJ. NO. EXPT. NO.

SUBJECT Continued from 42973-112

Continuation from 42973-112

2) Primary PCR using JNF1 and Q0(JNF4)

JNF1	1 ul
JNF4	1 ul
dNTP's	1 ul
10 x PCR Buffer	5 ul
~~T~~ cDNA	1 ul
dH_2O	39 ul
50x cDNA Advantage pigment	1 ul
	50 ul

PCR parameters: 94°C 1min 1cycle
94°C 1min
57°C 1min } 25cycles
72°C 2min
72°C 2min 1cycle

3) Secondary PCR using JNF2 and Q= (JNF5)

JNF2	1 ul
JNF5	1 ul
dNTP's	1 ul
10x PCR Buffer	5 ul
1/50 dilution of 1° PCR	1 ul
dH_2O	39 ul
50x Polymerase mix	1 ul
	50 ul

PCR parameters: Same as Primary PCR

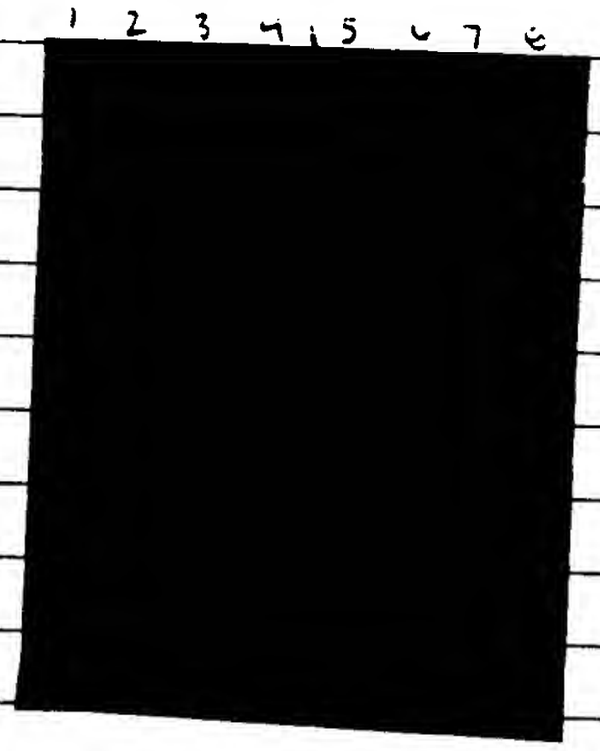
Continue to 114

SIGNED DATE WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DATE. ___ PROJ. NO. ___ EXPT. NO. ___

SUBJECT Continued from 42993-113



Lane 1 MW Marker
Lane 2 Dendritic 4-1
Lane 3 THP1 4-1
Lane 4 Jurkat 4-1
Lane 5 Dendritic 2-5
Lane 6 THP1 2-5
Lane 7 Jurkat 2-5
Lane 8 50bp Marker

Discussion

Lanes 2,3,4 on primary PCR using gspi(JNF1) and primer Qo(JNF4). This seems to be background products. Either extension time of 2 min was too long or Tm of annealing was low. Secondary PCR using gsp2(JNF2) and primer Qi(JNF5) reduced background considerably, but contains several bands which may be nonspecific(Lanes 5,6,7). Continue with RACE experiments, starting with 1/50 dilution of 1° PCR, but increase Tm to 58°C and reduce extension to 1min(since most prominent band in Jurkat Cine is around 900 bp).

END

SIGNED John N. Jin DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY. Sue Haber DATE

DO NOT WRITE IN THIS MARGIN

DATE: PROJ. NO. EXPT. NO.

SUBJECT Cloning of Full length DC54 cDNA including 3'-UTR.

Purpose: Isolate The full length DC54 cDNA of size approximately 2.6 Kb from 50 ul of PCR mixture.

5 Materials and Methods

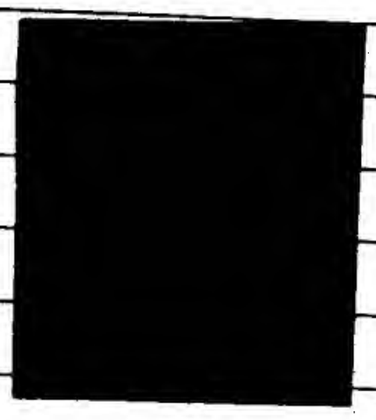
PCR

EP (Liewell-gal)	2.5 ul
RP (JNF4)	1.0 ul
dNTP's	1.0 ul
10x PCR Buffer	5.0 ul
Advantage Polymerase	1.0 ul
dH_2O	38.5 ul
Dendritic cDNA	1.0 ul
	50.0 ul

PCR parameters

94°C 1 min
94°C 1 min
57°C 1 min } 30
72°C 2.5 min
72°C 1.0 min

Run all 50 ul on 1.2% agarose gel.
Isolate 2.5 kb band using gel extraction kit (QIAgen)
Run on 2.0% gel; check conc.



lane 1: λØ marker.
lane 2: 1 ul isolated PCR fragment
lane 3: 3 ul isolated PCR fragment
lane 4: 2 ul Ø marker (1.3 kb band = 16 ng)
lane 5: 4 ul Ø marker (1.3 kb band = 32 ng)

Conc. of isolated fragment is 100 : ≈2 ng/ul

Ligate fragment into TA cloning vector

Results: Amount of 2.5kb fragment isolated is very low, but I ligated just to try and isolate a clone.

Discussion: No discussion yet, wait until transformation.

SIGNED DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: DATE

NO. 42973-160

BRISTOL–MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE: ______ PROJ. NO. ______ EXPT. NO. ______

SUBJECT: RT-PCR using primers JNF6 and JNF7

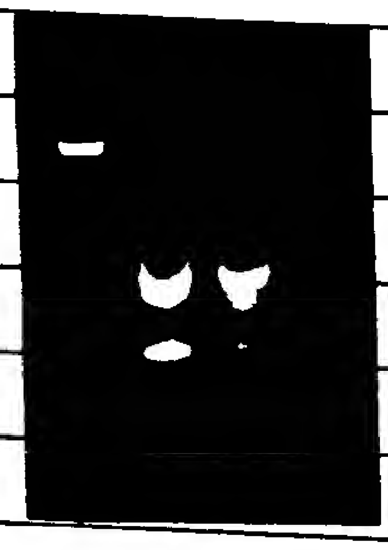
Purpose: To optimize parameters for RT-PCR expt., so as to get one band at 600 bp amplified in cell lines which express the DCSY gene.

Materials and Methods

i) 1st PCR:

JNF6 primer	1 ul	cDNA's used:
JNF7 primer	1 ul	a) Dendritic
dNTP's	1 ul	b) LPS Non-T cell
10x PCR Buffer	5 ul	c) THP1
Advantage Polymerase	1 ul	
dH_2O	40 ul	
DNA	1 ul	
	50 ul	

Parameters: 94°C 1 min
94°C 1 min
61°C 1 min } 30 cycles
72°C 45 sec
72°C 1 min



Lane 1: λ/φ marker
Lane 2: Dendritic
Lane 3: LPS NonT
Lane 4: THP1

RESULT 1: 600 bp band was evident, but a high background was present in reactions. Redo PCR with elevation of annealing temp to 63°C, in order to reduce background.

2) 2nd PCR:

JNF6 primer	0.5 ul	cDNA's used:
JNF7 primer	0.5 ul	a) LPS NonT ~~Dendritic~~
dNTP's	0.5 ul	b) THP1
10x PCR Buffer	2.5 ul	
Advantage Polymerase	0.5 ul	
dH_2O	20.0 ul	
cDNA	0.5 ul	
	25.0 ul	

Parameters: 94°C 1 min
94°C 1 min
63°C 1 min } 25 cycles
72°C 45 sec
72°C 1 min

continue 42973-129

SIGNED ______ DATE ______

WITNESSED AND UNDERSTOOD BY: ______ DATE ______

CROSS REFERENCES:

DATE PROJ. NO. EXPT. NO.

SUBJECT Cont'd 42973-128

Result 2: Significant reduction of background, paving conclusive identification of DC54 band in LPS Non T cell and no band in THP1 as found earlier in expt 42973-125.

Discussion: This expt. was done to optimize PCR parameter in RT-PCR of immunological cell lines in order to identify which cell type express the DC54 transcript. As shown earlier, LPS Non T cells express DC54, but THP1 does not. The significance of lack of expression in THP1 mRNA since the original subtraction exploited cDNA's expressed differentially between GMCSF/IL4 differentiated human monocytes and THP1 monocytes. This is also proven by expression of DC54 in dendritic cDNA. Repeat PCR for all available cell lines.

END

SIGNED DATE

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: DATE

DO NOT WRITE IN THIS MARGIN

NO. 42913-100

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE. PROJ. NC. EXPT. NC.

SUBJECT Full length cDNA sequence and a.a. translation for DC54

Word Document
FLDC54.doc

```
                                                GGAAGTGGCTTCATTTCAGTGGCTGACTTCCAGAGAGCAAT   41
ATGGCTGGTTCCCCAACATGCCTCACCCTCATCTATATCCTTTGGCAGCTCACAGGGTCA  101
M  A  G  S  P  T  C  L  T  L  I  Y  I  L  W  Q  L  T  G  S    20

GCAGCCTCTGGACCCGTGAAAGAGCTGGTCGGTTCCGTTGGTGGGGCCGTGACTTTCCCC  161
A  A  S  G  P  V  K  E  L  V  G  S  V  G  G  A  V  T  F  P    40

CTGAAGTCCAAAGTAAAGCAAGTTGACTCTATTGTCTGGACCTTCAACACAACCCCTCTT  221
L  K  S  K  V  K  Q  V  D  S  I  V  W  T  F  N  T  T  P  L    60

GTCACCATACAGCCAGAAGGGGGCACTATCATAGTGACCCAAAATCGTAATAGGGAGAGA  281
V  T  I  Q  P  E  G  G  T  I  I  V  T  Q  N  R  N  R  E  R    80

GTAGACTTCCCAGATGGAGGCTACTCCCTGAAGCTCAGCAAACTGAAGAAGAATGACTCA  341
V  D  F  P  D  G  G  Y  S  L  K  L  S  K  L  K  K  N  D  S   100

GGGATCTACTATGTGGGGATATACAGCTCATCACTCCAGCAGCCCTCCACCCAGGAGTAC  401
G  I  Y  Y  V  G  I  Y  S  S  S  L  Q  Q  P  S  T  Q  E  Y   120

GTGCTGCATGTCTACGAGCACCTGTCAAAGCCTAAAGTCACCATGGGTCTGCAGAGCAAT  461
V  L  H  V  Y  E  H  L  S  K  P  K  V  T  M  G  L  Q  S  N   140

AAGAATGGCACCTGTGTGACCAATCTGACATGCTGCATGGAACATGGGGAAGAGGATGTG  521
K  N  G  T  C  V  T  N  L  T  C  C  M  E  H  G  E  E  D  V   160

ATTTATACCTGGAAGGCCCTGGGGCAAGCAGCCAATGAGTCCCATAATGGGTCCATCCTC  581
I  Y  T  W  K  A  L  G  Q  A  A  N  E  S  H  N  G  S  I  L   180

CCCATCTCCTGGAGATGGGGAGAAAGTGATATGACCTTCATCTGCGTTGCCAGGAACCCT  641
P  I  S  W  R  W  G  E  S  D  M  T  F  I  C  V  A  R  N  P   200

GTCAGCAGAAACTTCTCAAGCCCCATCCTTGCCAGGAAGCTCTGTGAAGGTGCTGCTGAT  701
V  S  R  N  F  S  S  P  I  L  A  R  K  L  C  E  G  A  A  D   220

GACCCAGATTCCTCCATGGTCCTCCTGTGTCTCCTGTTGGTGCCCCTCCTGCTCAGTCTC  761
D  P  D  S  S  M  V  L  L  C  L  L  L  V  P  L  L  L  S  L   240

TTTGTACTGGGGCTATTTCTTTGGTTTCTGAAGAGAGAGAGACAAGAAGAGTACATTGAA  821
F  V  L  G  L  F  L  W  F  L  K  R  E  R  Q  E  E  Y  I  E   260

GAGAAGAAGAGAGTGGACATTTGTCGGGAAACTCCTAACATATGCCCCCATTCTGGAGAG  881
E  K  K  R  V  D  I  C  R  E  T  P  N  I  C  P  H  S  G  E   280

AACACAGAGTACGACACAATCCCTCACACTAATAGAACAATCCTAAAGGAAGATCCAGCA  941
N  T  E  Y  D  T  I  P  H  T  N  R  T  I  L  K  E  D  P  A   300

AATACGGTTTACTCCACTGTGGAAATACCGAAAAAGATGGAAAATCCCCACTCACTGCTC 1001
N  T  V  Y  S  T  V  E  I  P  K  K  M  E  N  P  H  S  L  L   320

ACGATGCCAGACACACCAAGGCTATTTGCCTATGAGAATGTTATCTAGACAGCAGTGCAC 1061
T  M  P  D  T  P  R  L  F  A  Y  E  N  V  I  *               335

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CCCTATTGTAGTAAAAAAGTCTTCTTTACTATCTTAATAAAACAGATATTGTGAGATTCA 2681
CATAAAAAAAAAAAAAAAAAAAA
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SIGNED DATE

CROSS REFERENCES

WITNESSED AND UNDERSTOOD BY: DATE